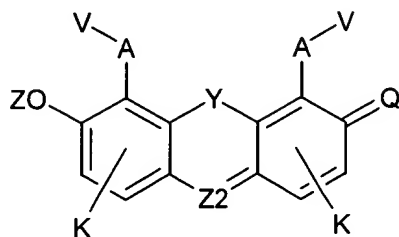
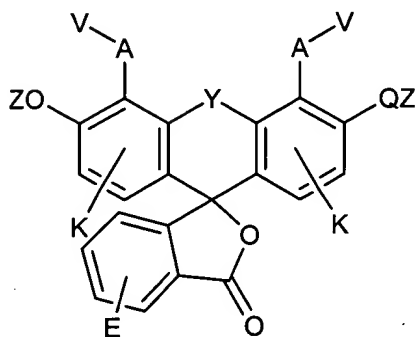


In the claims:

1. **(currently amended)** A fluorescein-based ligand, comprising a ligand having one of the following structures:

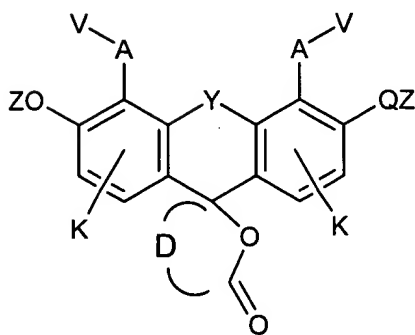


I



II

or



III

wherein, independently for each occurrence:

A is  $-\text{CH}_2-$ ,  $-\text{C}(=\text{O})-$ ,  $-\text{C}(=\text{S})-$ ,  $-\text{CH}_2\text{CH}_2-$ ,  $-\text{CH}_2\text{C}(=\text{O})-$ ,  $-\text{CH}_2\text{C}(=\text{S})-$  or  $-\text{C}(\text{H})=$ ;

Z is hydrogen or any hydroxyl-protecting group;

Q is O, S or Se;

K is optionally present and if present, is any one or more of the following substituents at one or more of the substitutable positions of the indicated aromatic ring: alkyl, alkenyl, alkynyl, amineo, acyl, acyloxy, acylamino, alkylthio, alkoxyl, nitro, halogen, sulfhydryl, cyano, hydroxyl, carbamoyl and trifluoromethyl;

V is (i) a chemical moiety comprising at least three Lewis basic moieties each independently selected from the group of Lewis basic moieties consisting of: amino, amido, nitro, nitroso, amino alcohol, nitrile, imino, isonitrile, cyanate, isocyanate, phosphate, phosphonate, phosphite, phosphine, phosphine oxide, phosphorothioate, phosphoramidate, phosphonamidite, hydroxyl, carbonyl, aldehyde, ketone, ether, carbamoyl, thiol, sulfide, thiocarbonyl, thioether, mercaptan, sulfonic acid, sulfoxide, sulfate, sulfonate, sulfone, sulfonamide, sulfamoyl, sulfinyl, or heterocyclyl, wherein the at least three Lewis basic moieties are capable of forming a tridentate chelate and at least one of the Lewis basic moieties is heterocyclyl or (ii) an imino group, wherein said imino group is capable of forming a bidentate chelate;

Y is O, S, Se, NR, or  $\text{C}(\text{CH}_3)_2$ , wherein R is an alkyl and R and the methyl groups of  $\text{C}(\text{CH}_3)_2$  are optionally substituted;

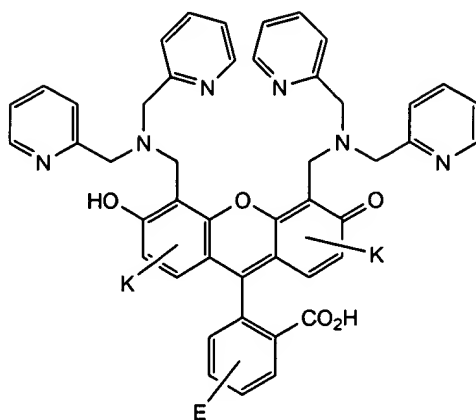
Z2 is N,  $\text{HOOCCH}_2\text{CH}_2\text{C}-$ ,  $\text{HOOC}-\text{CH}=\text{CH}-\text{C}-$ , (2-carboxyphenyl)-C-, or (2-sulfophenyl)-C-, wherein for said (2-carboxyphenyl)-C- and (2-sulfophenyl)-C-, said phenyl moiety is optionally substituted with one or more E, and wherein for said  $\text{HOOCCH}_2\text{CH}_2\text{C}-$  and  $\text{HOOC}-\text{CH}=\text{CH}-\text{C}-$ , said hydrogen atoms of said  $-\text{CH}_2-$ 's and  $-\text{CH}=$ 's moieties are optionally substituted;

E is optionally present and if present, is any one or more of the following substituents at one or more of the substitutable positions of the indicated aromatic ring: alkyl, alkenyl, alkynyl, amino, acyl, acyloxy, acylamino, alkylthio, carbonyl, alkoxyl, sulfonyl, nitro, halogen, sulfhydryl, cyano, hydroxyl, carbamoyl and trifluoromethyl; and

D is  $-\text{CH}_2\text{CH}_2-$  or  $-\text{CH}=\text{CH}-$ , wherein said hydrogen atoms are optionally substituted.

2. **(previously presented)** The fluorescein-based ligand of claim 1, wherein A is  $-CH_2-$ , Y is O, and Q is O.
3. **(currently amended)** The fluorescein-based ligand of claim 2, wherein: said ligand has formula I or II; K is optionally present and if present, is any one or more of the following substituents of the indicated aromatic ring: halogen; Z2 is (2-carboxyphenyl)-C-; and E is optionally present and if present, is any one or more of the following substituents of the indicated aromatic ring: amino, nitro, ~~carbonyl~~ and halogen.
4. **(previously presented)** The fluorescein-based ligand of claim 3, wherein K is present as halogen in either or both of the 2' and 7' positions of said ligand, and wherein E is not present.
5. **(previously presented)** The fluorescein based-ligand of claim 1, wherein: Q is O; Y is O; and one V is amino.
6. **(previously presented)** The fluorescein-based ligand of claim 1, wherein: Y is O; Q is O; Z is H; and one V is amino.
7. **(previously presented)** The fluorescein-based ligand of claim 3, wherein K is present at both the 2' and 7' positions of said ligand.
8. **(currently amended)** The fluorescein-based ligand of claim 4, wherein: said ligand has formula I or II; K is optionally present and if present, is any one or more of the following substituents of the indicated aromatic ring: halogen; Z2 is (2-carboxyphenyl)-C-; and E is optionally present and if present, is any one or more of the following substituents of the indicated aromatic ring: amino, nitro, ~~carbonyl~~ and halogen.
9. **(previously presented)** The fluorescein-based ligand of claim 8, wherein K is present in one or both of the indicated aromatic rings as halogen.
10. **(currently amended)** The fluorescein-based ligand of claim 8, wherein E is present in said ligand as ~~either one carbonyl or one amino~~.

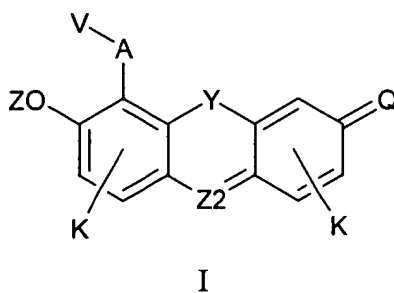
11. **(previously presented)** The fluorescein-based ligand of claim 1, wherein said ligand has the following structure:

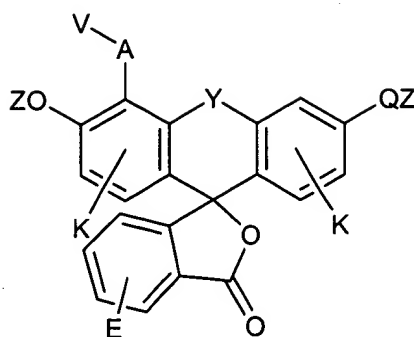


wherein K is optionally present and if present, is any one or more of the following substituents of the indicated aromatic ring: halogen.

Claims 12-19 (**canceled**)

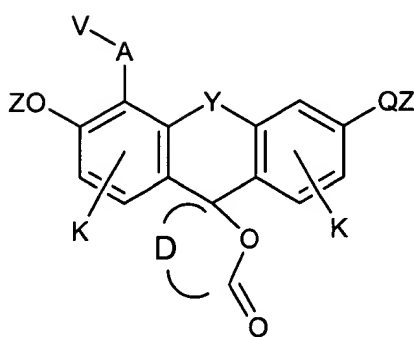
20. **(currently amended)** A fluorescein-based ligand, comprising a ligand having one of the following structures:





II

or



III

wherein, independently for each occurrence:

A is  $-\text{CH}_2-$ ,  $-\text{C}(=\text{O})-$ ,  $-\text{C}(=\text{S})-$ ,  $-\text{CH}_2\text{CH}_2-$ ,  $-\text{CH}_2\text{C}(=\text{O})-$ ,  $-\text{CH}_2\text{C}(=\text{S})-$  or  $-\text{C}(\text{H})=$ ;

Z is hydrogen or any hydroxyl-protecting group;

Q is O, S or Se;

K is optionally present and if present, is any one or more of the following substituents at one or more of the substitutable positions of the indicated aromatic ring: alkyl, alkenyl, alkynyl, amino, acyl, acyloxy, acylamino, alkylthio, alkoxyl, nitro, halogen, sulfhydryl, cyano, hydroxyl, carbamoyl and trifluoromethyl;

V is (i) a chemical moiety comprising at least three Lewis basic moieties each independently selected from the group of Lewis basic moieties consisting of: amino, amido, nitro, nitroso, amino alcohol, nitrile, imino, isonitrile, cyanate, isocyanate, phosphate, phosphonate, phosphite, phosphine, phosphine oxide, phosphorothioate, phosphoramidate, phosphoramidite, hydroxyl, carbonyl, aldehyde, ketone, ether, carbamoyl, thiol, sulfide, thiocarbonyl, thioether, mercaptan, sulfonic acid, sulfoxide,

sulfate, sulfonate, sulfone, sulfonamide, sulfamoyl, sulfinyl, or heterocyclyl, wherein the at least three Lewis basic moieties are capable of forming a tridentate chelate and at least one of the Lewis basic moieties is heterocyclyl or (ii) an imino group, wherein said imino group is capable of forming a bidentate chelate;

Y is O, S, Se, NR, or C(CH<sub>3</sub>)<sub>2</sub>, wherein R is an alkyl and R and the methyl groups of C(CH<sub>3</sub>)<sub>2</sub> are optionally substituted;

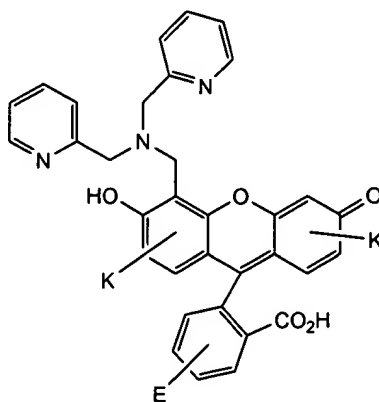
Z2 is N, HOOCCH<sub>2</sub>CH<sub>2</sub>C-, HOOC-CH=CH-C-, (2-carboxyphenyl)-C-, or (2-sulfophenyl)-C-, wherein for said (2-carboxyphenyl)-C- and (2-sulfophenyl)-C-, said phenyl moiety is optionally substituted with one or more E, and wherein for said HOOCCH<sub>2</sub>CH<sub>2</sub>C- and HOOC-CH=CH-C-, said hydrogen atoms of said -CH<sub>2</sub>-’s and -CH=’s moieties are optionally substituted;

E is optionally present and if present, is any one or more of the following substituents at one or more of the substitutable positions of the indicated aromatic ring: alkyl, alkenyl, alkynyl, amino, acyl, acyloxy, acylamino, alkylthio, ~~carbonyl~~, alkoxyl, ~~sulfonyl~~, nitro, halogen, sulfhydryl, cyano, hydroxyl, carbamoyl and trifluoromethyl; and

D is -CH<sub>2</sub>CH<sub>2</sub>- or -CH=CH-, wherein said hydrogen atoms are optionally substituted.

21. **(previously presented)** The fluorescein-based ligand of claim 20, wherein A is -CH<sub>2</sub>-, optionally substituted, Y is O, and Q is O.
22. **(currently amended)** The fluorescein-based ligand of claim 21, wherein: said ligand has formula I or II; K is optionally present and if present, is any one or more of the following substituents of the indicated aromatic ring: halogen; Z2 is (2-carboxyphenyl)-C-; and E is optionally present and if present, is any one or more of the following substituents of the indicated aromatic ring: amino, nitro, ~~carbonyl~~ and halogen.
23. **(previously presented)** The fluorescein-based ligand of claim 22, wherein K is present as halogen in either or both of the 2’ and 7’ positions of said ligand, and wherein E is not present.

24. **(previously presented)** The fluorescein based-ligand of claim 20, wherein: Q is O; Y is O; and one V is amino.
25. **(previously presented)** The fluorescein-based ligand of claim 20, Y is O; Q is O; Z is H; and one V is amino.
26. **(previously presented)** The fluorescein-based ligand of claim 22, wherein K is present at both the 2' and 7' positions of said ligand.
27. **(currently amended)** The fluorescein-based ligand of claim 24, wherein: said ligand has formula I or II; K is optionally present and if present, is any one or more of the following substituents of the indicated aromatic ring: halogen; Z2 is (2-carboxyphenyl)-C-; and E is optionally present and if present, is any one or more of the following substituents of the indicated aromatic ring: amino, nitro, ~~carbonyl~~ and halogen.
28. **(previously presented)** The fluorescein-based ligand of claim 27, wherein K is present in one or both of the indicated aromatic rings as halogen.
29. **(currently amended)** The fluorescein-based ligand of claim 27, wherein E is present in said ligand as ~~either one carbonyl or~~ one amino.
30. **(previously presented)** The fluorescein-based ligand of claim 20, wherein said ligand has the following structure:

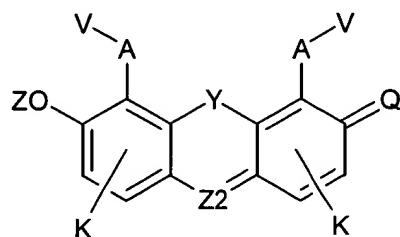


wherein K is optionally present and if present, is any one or more of the following substituents of the indicated aromatic ring: halogen.

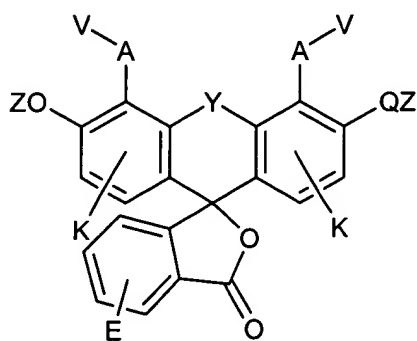
Claims 31-47 (**canceled**)

48. **(currently amended)** A diagnostic kit for a metal ion, comprising:

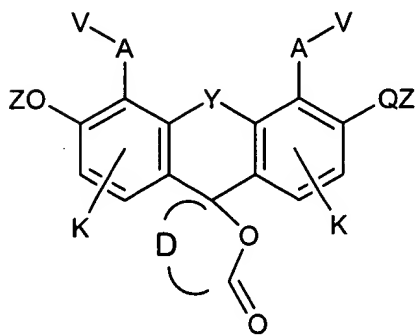
a. A fluorescein-based ligand comprising one of the following structures:



I

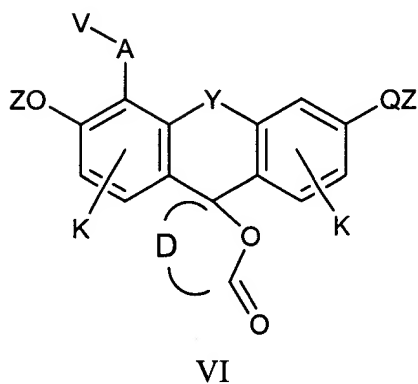
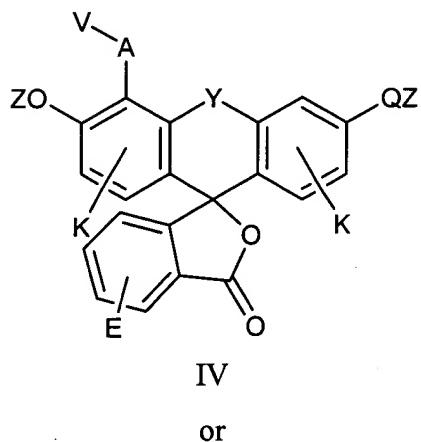
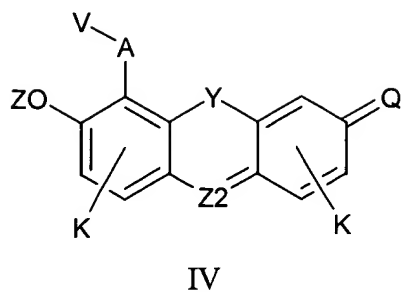


II



III





wherein, independently for each occurrence:

A is  $-\text{CH}_2-$ ,  $-\text{C}(=\text{O})-$ ,  $-\text{C}(=\text{S})-$ ,  $-\text{CH}_2\text{CH}_2-$ ,  $-\text{CH}_2\text{C}(=\text{O})-$ ,  $-\text{CH}_2\text{C}(=\text{S})-$  or  $-\text{C}(\text{H})=$ ;

Z is hydrogen or any hydroxyl-protecting group;

Q is O, S or Se;

K is optionally present and if present, is any one or more of the following substituents at one or more of the substitutable positions of the indicated aromatic ring:

alkyl, alkenyl, alkynyl, amino, acyl, acyloxy, acylamino, alkylthio, alkoxyl, nitro, halogen, sulfhydryl, cyano, hydroxyl, carbamoyl and trifluoromethyl;

V is (i) a chemical moiety comprising at least three Lewis basic moieties each independently selected from the group of Lewis basic moieties consisting of: amino, amido, nitro, nitroso, amino alcohol, nitrile, imino, isonitrile, cyanate, isocyanate, phosphate, phosphonate, phosphite, phosphine, phosphine oxide, phosphorothioate, phosphoramidate, phosphonamidite, hydroxyl, carbonyl, aldehyde, ketone, ether, carbamoyl, thiol, sulfide, thiocarbonyl, thioether, mercaptan, sulfonic acid, sulfoxide, sulfate, sulfonate, sulfone, sulfonamide, sulfamoyl, sulfinyl, or heterocyclyl, wherein the at least three Lewis basic moieties are capable of forming a tridentate chelate and at least one of the Lewis basic moieties is heterocyclyl or (ii) an imino group, wherein said imino group is capable of forming a bidentate chelate;

Y is O, S, Se, NR, or  $C(CH_3)_2$ , wherein R is an alkyl and R and the methyl groups of  $C(CH_3)_2$  are optionally substituted;

Z2 is N,  $HOOCCH_2CH_2C-$ ,  $HOOC-CH=CH-C-$ , (2-carboxyphenyl)-C-, or (2-sulfophenyl)-C-, wherein for said (2-carboxyphenyl)-C- and (2-sulfophenyl)-C-, said phenyl moiety is optionally substituted with one or more E, and wherein for said  $HOOCCH_2CH_2C-$  and  $HOOC-CH=CH-C-$ , said hydrogen atoms of said  $-CH_2-$ 's and  $-CH=$ 's moieties are optionally substituted;

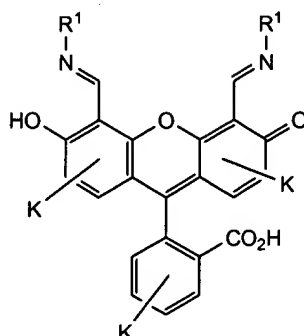
E is optionally present and if present, is any one or more of the following substituents at one or more of the substitutable positions of the indicated aromatic ring: alkyl, alkenyl, alkynyl, amino, acyl, acyloxy, acylamino, alkylthio, ~~carbonyl~~, alkoxyl, ~~sulfonyl~~, nitro, halogen, sulfhydryl, cyano, hydroxyl, carbamoyl and trifluoromethyl; and

D is  $-CH_2CH_2-$  or  $-CH=CH-$ , wherein said hydrogen atoms are optionally substituted; and

b. Instructions for using said ligand to detect a metal ion in a sample.

49. (canceled)

50. **(previously presented)** A fluorescein-based ligand, wherein said ligand has the following structure:

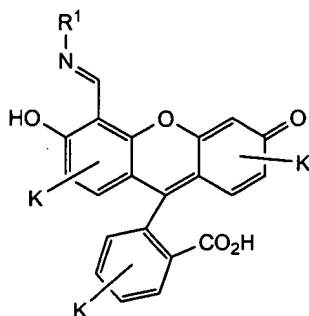


wherein, independently for each occurrence:

R¹ represents optionally substituted aliphatic, alkyl, aralkyl, alkenyl, alkynyl, aryl or heterocyclyl; and

K is optionally present and if present, is any one or more of the following substituents at one or more of the substitutable positions of the indicated aromatic ring: alkyl, alkenyl, alkynyl, amino, acyl, acyloxy, acylamino, alkylthio, alkoxyl, nitro, halogen, sulfhydryl, cyano, hydroxyl, carbamoyl and trifluoromethyl.

51. **(previously presented)** A fluorescein-based ligand, wherein said ligand has the following structure:



wherein

R¹ represents optionally substituted aliphatic, alkyl, aralkyl, alkenyl, alkynyl, aryl or heterocyclyl; and

K is optionally present and if present, is any one or more of the following substituents at one or more of the substitutable positions of the indicated aromatic ring: alkyl, alkenyl, alkynyl, amino, acyl, acyloxy, acylamino, alkylthio, alkoxyl, nitro, halogen, sulfhydryl, cyano, hydroxyl, carbamoyl and trifluoromethyl.